PA 1278456

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 01, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/515,697*
FILING DATE: *October 31, 2003*

1B/04/3559

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

M. K. HAWKINS
Certifying Officer

Please type a plus sign (+) inside this box → ☒

PTO/SB/16 (02-01)
Approved for use through10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Guobin	MA	Dorval, Canada
Frédéric	LESAGE	Montreal, Canada

☒ Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

RAPAMYCIN PEPTIDES CONJUGATES: SYNTHESIS AND USES THEREOF

Direct all correspondence to: CORRESPONDENCE ADDRESS

☒ Customer Number 020988 → Place Customer Number Bar Code Label here

OR Type Customer Number here

☐ Firm or Individual Name	OGILVY RENAULT				
Address	1981 McGill College Avenue				
Address	Suite 1600				
City	Montreal	State	Quebec	ZIP	H3A 2Y3
Country	Canada	Telephone	613-780-8661	Fax	613-230-6706

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 19		☐ CD(s), Number	
☒ Drawing(s) Number of Sheets 3		☒ Other (specify)	Fee Transmittal PTO/SB17
☒ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

		FILING FEE AMOUNT ($)
☐ Applicant claims small entity status. See 37 CFR 1.27.		
☐ A check or money order is enclosed to cover the filing fees		
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:	19-5113	160.00
☐ Payment by credit card. Form PTO-2038 is attached.		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date 31/ 10 / 03

TYPED or PRINTED NAME Wayne H. YAN REGISTRATION NO. (if appropriate) 44,485

TELEPHONE 613-780-8682 Docket Number: 15814-11USPR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (02-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Docket Number	15814-11USPR	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle (if any))	Family or Surname	Residence (City and either State or Foreign Country)
David	HALL	Montreal, Canada

Number __2__ of __2__

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 160

Complete If Known	
Application Number	
Filing Date	
First Named Inventor	MA, Guobin
Examiner Name	
Art Unit	
Attorney Docket No.	15186-46USPR WHY/LB/sw

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 19-5113

Deposit Account Name: Ogilvy Renault

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	160

SUBTOTAL (1) ($) 160

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** =		x =
Independent Claims		- 3** =		x =
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 0

SUBMITTED BY				(Complete if applicable)	
Name (Print/Type)	Wayne H. Yan	Registration No. (Attorney/Agent)	44,485	Telephone	613-780-8673
Signature				Date	October 31, 2003

WARNING: Information on this form may become public. Credit card information should not be included in this form. Provide credit card information and authorization on PTO-2038.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

APPLICATION INFORMATION

Application number::	
Filing Date::	
Application Type::	Provisional
Title::	A TIME-DOMAIN METHOD FOR DETERMINING THE DEPTH OF A FLUOROPHORE IN A TURBID MEDIUM AND ESTIMATING THE FLUROPHORE CONCENTRATION
Attorney Docket Number::	15186-46USPR
Total Drawing Sheets::	3
Small Entity?::	No

INVENTOR INFORMATION

Inventor Authority Type::	Inventor
Primary Citizenship Country::	China
Status::	Full Capacity
Given name::	Guobin
Middle name::	
Family name::	MA
Name Suffix::	
City of Residence::	Dorval
State or Province of Residence::	Quebec
Country of Residence::	Canada
Street::	307 Legace Avenue
City::	Dorval
State or Province::	Quebec
Country::	Canada
Postal or Zip Code::	H9S 2M2

Inventor Authority Type::	Inventor
Primary Citizenship Country::	Canadian
Status::	Full Capacity
Given name::	Frédéric
Middle name::	
Family name::	LESAGE
Name Suffix::	
City of Residence::	Montreal
State or Province of Residence::	Quebec
Country of Residence::	Canada

Street::	5192 Chabot
City::	Montreal
State or Province::	Quebec
Country::	Canada
Postal or Zip Code::	H2H 1Y8
Inventor Authority Type::	Inventor
Primary Citizenship Country::	Great Britain
Status::	Full Capacity
Given name::	David
Middle name::	
Family name::	HALL
Name Suffix::	
City of Residence::	Montreal
State or Province of Residence::	Quebec
Country of Residence::	Canada
Street::	#1005 - 3445 Rue Drummond
City::	Montreal
State or Province::	Quebec
Country::	Canada
Postal or Zip Code::	H3G 1X9

CORRESPONDENCE INFORMATION

Correspondence Customer Number::	020988
Phone number::	(514) 845-7126
Fax::	(514) 288-8389
E-Mail Address::	swabey@ogilvyrenault.com

REPRESENTATIVE INFORMATION

Representative Customer Number:: 020988

ASSIGNEE INFORMATION

Assignee name::	ART Recherches et Technologies Avancées Inc.
Street::	2300 Blvd. Alfred-Nobel
City::	St-Laurent
State or Province::	Quebec
Country::	Canada
Postal or Zip Code::	H4S 2A4

OGILVY
RENAULT

Direct Dial: (613) 780-8601
Direct Fax: (613) 230-6706
lberube@ogilvyrenault.com

Ottawa, October 31, 2003

Mail Stop Provisional Patent Application

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
U.S.A.

Sir:

Re: New United States Provisional Patent Application
Title: A TIME-DOMAIN METHOD FOR DETERMINING THE DEPTH OF A FLUOROPHORE IN A TURBID MEDIUM AND ESTIMATING THE FLUROPHORE CONCENTRATION
Inventors: MA, Guobin, LESAGE, Frédéric and HALL, David
Our File: 15186-46USPR WHY/LB/sw

Transmitted herewith for filing is the provisional patent application of:

INVENTORS: **MA, Guobin**
LESAGE, Frédéric
HALL, David

TITLE OF THE INVENTION:

A TIME-DOMAIN METHOD FOR DETERMINING THE DEPTH OF A FLUOROPHORE IN A TURBID MEDIUM AND ESTIMATING THE FLUROPHORE CONCENTRATION

The following documents are enclosed:

a) Fee Transmittal Form PTO/SB/17 (in duplicate) (2 pgs.) for Filing Fee of $160.00 (Deposit Acct. 19-5113)

OGILVY
RENAULT

b) Application Data Sheet (3 pgs.)

c) Provisional application cover sheet (2 pgs.)

d) Specification and 10 claims (19 pgs.)

The Commissioner is hereby authorized to charge the filing fee and any additional fees which may be required, or credit any overpayment to our Account No. 19-5113.

Please note that the address of the agents for the applicant in this matter should be the Montreal address identified in the enclosed Provisional Application Cover Sheet. Correspondence may be directed to Wayne H. Yan at the Montreal address of Ogilvy Renault.

Respectfully submitted,

Wayne H. Yan
Reg. No. 44,485
Agent of Record

WHY/LB/sw
Encls.

A TIME-DOMAIN METHOD FOR DETERMINING THE DEPTH OF A FLUOROPHORE IN A TURBID MEDIUM AND ESTIMATING THE FLUROPHORE CONCENTRATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is the first application filed for the present invention.

TECHNICAL FIELD

[0002] This application relates to fluorescence measurements in turbid media and more specifically to fluorescence measurements using the time domain optical method.

BACKGROUND OF THE INVENTION

[0003] Optical fluorescence imaging of turbid media, e.g. biological tissue, is primarily achieved with Continuous Wave (CW) methods. Typically a light source is employed to illuminate the object of interest, e.g. a mouse in vivo, and the emitted CW fluorescence intensity signal from the flurophore is measured directly with a CCD camera, e.g. AntiCancer Inc.

[0004] In this context, it is often useful to determine the concentration and depth of the fluorophore. However, to assume that the direct CW fluorescence intensity signal is proportional to the flurophore concentration can be misleading since the depth of the flurophore will also impact the CW fluorescence intensity signal. In fact, given a single CW source and CW detector measurement it is impossible to decouple flurophore concentration and depth.

[0005] To address this problem of decoupling depth and concentration in CW measurements, some researchers are exploring CW tomography, in order to reconstruct a three-dimensional distribution of the flurophore concentration in the object, thereby attempting to account for the depth of the flurophore. However, these tomographic approaches require multiple source-detector pair measurements from many angles, combined with complex, computer intensive inversion algorithms. Furthermore, CW tomography requires an assumption about the scattering coefficient of the object, since CW can neither decouple the intrinsic absorption and scattering of the object.

[0006] The most complete description of photon migration in turbid media is provided by Time Domain (TD) optical methods which have previously been used to decouple the attenuation coefficient, given from CW intensity measurements, into the underlying absorption and scattering coefficients. However TD methods have not been applied to obtain depth and concentration of fluorophores.

SUMMARY OF THE INVENTION

[0007] The invention relates to the measurement of fluorophores position and concentration within a turbid medium. More specifically the invention relates to the measurement of fluorophores position and concentration within a turbid medium using Time Domain (TD) optical method.

[0008] There is provided a method wherein TD optical methods are used to decouple fluorophore depth and

concentration from a single source and detector measurement employing a direct analysis technique, thereby avoiding the aforementioned burden of CW tomography and overcoming the limitations of conventional CW direct fluorescence intensity imaging.

[0009] In one embodiment there is provided method for determining the depth of a volume comprising a fluorophore in a turbid medium using time domain(TD) optical fluorescence by obtaining Temporal Point Spread Function (TPSF), data corresponding to at least one TPSF, by injecting light at an injection point at an excitation wavelength of the fluorophore and detecting light at a detection point at an emission wavelength of the fluorophore, the injection and detection points being in a reflection geometry and substantially equidistant from said volume, determining a time (t_{max}) substantially corresponding to the maximum of the TPSF and correlating t_{max} with the depth.

[0010] In another embodiment there is also provided a method for estimating the concentration of a fluorophore in a volume of a turbid medium using optical fluorescence by obtaining depth of the volume, providing optical properties for the medium, obtaining a CW intensity surface reflection measurement of the fluorophore, and normalizing the CW intensity measurements using optical properties of the medium and the fluorophore depth to obtain a relative fluorophore concentration and/or absolute concentration.

[0011] In yet a further embodiment there is provided a method for generating a tomographic image of a fluorophore distribution in a turbid medium by obtaining a topographic

image of the fluorophore distribution, determining the depth of a plurality of volumes of interest comprising the fluorophore using depth determination methods of the present invention, and combining the depth information and the topographic image to generate a tomographic image of the fluorophore distribution.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Further features and advantages of the present invention will become apparent from the following detailed description, taken in combination with the appended drawings, in which:

[0013] Fig. 1 is a schematic representation of a preferred fluorescence signal acquisition;

[0014] Fig. 2 is a 3-dimensional graphic relating t_{max}, depth of fluorophore and the concentration of the fluorophore; and

[0015] Fig. 3 is a 3-dimensional graphic relating CW signal intensity, depth of fluorophore and the concentration of the fluorophore.

[0016] It will be noted that throughout the appended drawings, like features are identified by like reference numerals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0017] In the following description it will be appreciated that CW intensity can be obtained using direct CW measurements or by integrating a Temporal Point Spread Function (TPSF) obtained by the time domain (TD) method.

[0018] It will also be appreciated that the term fluorophore can refer to either an extrinsic fluorophore which is understood to be a fluorophore that is added to a given medium or an intrinsic fluorophore which is understood to be a fluorophore that is normally comprised in a given medium. For example biological tissue may comprise molecules that naturally fluoresce and are therefore intrinsic fluorophores.

[0019] The fluorescence emission from a fluorophore embedded in a turbid medium can be measured over time using time domain optical fluorescence to generate Temporal Point Spread Function (TPSF) data. Figure 1 depicts a preferred arrangement used to obtained the TPSF. One or more fluorophore is included in a turbid medium and excitation light is injected at injection point 10. The photons diffuse and a certain fraction of the photons eventually reach the fluorophore molecules contained in a volume of interest (VOI) 14 which are thereby excited. The molecules then emit a fluorescence signal. The emission photons will diffuse and a fraction of them will reach detection point 12 to produce TPSF data.

[0020] TD fluorescence experiments were conducted with a single source-detector measurement for a small inclusion of fixed flurophore concentration submerged into a turbid, liquid medium. It was observed that the temporal position of the maximum of the TPSF generated by the fluorescent photons (t_{max}) increased with the depth of the submersion of the inclusion in the turbid medium (see figure 1).

[0021] TD fluorescence experiments were also conducted with a single source-detector measurement for small

inclusions of various fluorophore concentrations submerged at fixed depths in a turbid, liquid medium. It was observed that t_{max} generated by the fluorescent photons remained constant for inclusions of the same submersion depth, regardless of their fluorophore concentration (also shown in Figure 2). Hence it was demonstrated that t_{max} from a fluorophore inclusion permits decoupling of its depth from its concentration.

[0022] For the above series of experiments CW data was also generated by temporally integrating the complete fluorescent TPSF. As expected, the CW fluorescence intensity decreased as the depth of the fixed fluorophore concentration increased, but it also decreased as the fluorophore concentration of inclusions at a fixed depth decreased, as shown in Figure 3. Hence, it was demonstrated that direct CW fluorescence intensity measurements alone cannot decouple fluorophore inclusion concentration from depth.

[0023] In a preferred embodiment the acquisition of the fluorescence signal is performed in the reflective mode, that is to say, the injection and detection points are substantially equidistant from the volume comprising the fluorophore.

[0024] Thus in one embodiment of the invention the depth of a volume comprising a fluorophore can be determined by establishing a calibration curve for which t_{max} measurements are obtained for known depth of the volume and using this calibration curve to estimate the depth of a volume which is originally unknown.

[0025] It will be appreciated that the depth of more than one fluorophore can be assessed by detecting a different emission wavelength for each of the fluorophore provided that their emission spectra do not overlap.

[0026] In another aspect of the present invention there is provided a method wherein an analytical relationship between t_{max} and optical characteristics of the medium is used to calculate the depth of the fluorophore.

[0027] Under certain assumptions such as assuming that the optical properties of the medium are the same at the excitation and emission wavelength, the fluorescence intensity as a function of time can be expressed by the Born approximation:

[0028] $$\phi(t) \cong \sum_{dipoles} \left(QC \frac{r_{sp} + r_{pd}}{4\pi D r_{sp} r_{pd}} v (4\pi D v t)^{-3/2} e^{\frac{(r_{sp}+r_{pd})^2}{4Dvt} - \mu_a v t} \right) * \left(\frac{e^{-\frac{t}{\tau}}}{\tau} \right) * (impulse\,Response)$$

(equation 1)

[0029] Where:

[0030] r_{sp} is the distance from source (s) to fluorophore depth position (p);

[0031] r_{pd} is the distance from fluorophore depth position (p) to detector (d);

[0032] μ_a is the optical absorption coefficient;

[0033] D is the optical diffusion coefficient; and

[0034] v is the speed of light in the medium

[0035] By setting the first derivative of equation 1 as a function of time equal to zero, the maximum of the TPSF (t_{max}) can be found. Under certain approximations, and by assuming that r_{sp} is approximately equal to r_{ps}, it is found that:

[0036] $$t_{max} \cong \frac{d\sqrt{\tau}}{\sqrt{Dv}} \qquad \text{(equation 2)}$$

[0037] where d is the depth of the fluorophore, $D = \frac{1}{3\mu_s'}$.

It will be appreciated that t_{max}, or any time point between the earliest detected signal (time "0") up to and including t_{max} (and therefore d) can be more accurately determined numerically using the full equation 1.

[0038] As can be seen the depth of the fluorophore is proportional to t_{max}. In order to determine the depth of a volume comprising a fluorophore in a turbid medium, the speed of light and the scatter coefficient can be provided using known values for the medium. Alternatively, the scattering coefficent can be provided using the standard time domain approach by direct measurements.

[0039] The scattering and absorption coefficients can be determined at either the excitation and/or emission wavelengths of the fluorophore, since in a preferred embodiment the method assumes that these values are the same for the excitation and emission wavelengths. However, it is also possible to derive t_{max} by using the scattering and absorption coefficients as determined at both the excitation and emission wavelengths of the fluorophore. In this respect, it will be appreciated that a more general form of equation 1 in which the absorption and scatter

coefficients, determined at both the emission and excitation wavelengths, are used is also encompassed in the present invention.

[0040] The calculation is based in part on the assumption that the distance between the point of light injection at the surface and the fluorophore and between the fluorophore and the point of fluorescence emission detection at the same surface is substantially the same (figure 1). Determination of the injection and detection points relative to the volume of interest for this reflection geometry can be achieved, for example by obtaining a topographic image of the fluorophore in a region of interest. In this respect the 2D (topographic) image may be obtained by optical modalities such as TD, CW and frequency domain (FD).

[0041] Once the injection and detection points have been determined, TPSF data is then acquired by injecting pulses of light at an excitation wavelength of the fluorophore at the injection point and by detecting as a function of time the fluorescence emission at an emission wavelength. TPSF generation using TD optical method are well known in the art. The time at which the TPSF reaches a maximum (t_{max}) is then recorded and used in the above relationships (either equation 1 or equation 2) to determine the depth of the fluorophore.

[0042] It will be appreciated that a single injection/detection points pair is sufficient to determine the depth, therefore providing a rapid method for determination of depth. However, it is also possible to use

multiple injection/detection pairs to improve the accuracy of depth determination.

[0043] For a given depth, the surface CW intensity can be related to fluorophore concentration by the optical properties of the medium (absorption and scattering coefficients). Thus, in another aspect of the invention, estimates of the relative concentration of the fluorophore, $Conc._{Relative}$, can be obtained by determining its depth, d, using the method described above, and normalizing the surface CW intensity measurement, CW, as follows (Equation 3):

[0044] $$Conc._{Relative} = CWd^2 e^{2d\sqrt{\mu_a / D}}$$ (equation 3)

[0045] under certain assumptions, Equation 3 can be derived from equation 1.

[0046] This method provide a means for rapidly compare the relative concentration of a fluorophore at 2 or more distinct locations. The determination of the relative concentration of a fluorescently labeled antibody in different organs of an animal provides a non-limiting example.

[0047] Relative concentration can be further processed to estimates of absolute concentration if the CW surface measurement is absolutely calibrated. That is to say a calibration curve can be established for CW using known concentration of the fluorophore. The scattering and abosrption coefficent can be provided using known values for the medium or alternatively may be provided using the standard time domain approach by direct measurements. It will also be appreciated that the CW surface intensity may

be provided by temporally integrating the time domain fluorescent measurement.

[0048] By further analyzing the CW fluorescence intensity data from the above experiments, it was possible to calibrate the optical attenuation of the turbid medium. In other words, for the inclusion of fixed fluorophore concentration, the optical attenuation can be obtained by fitting the decrease of CW fluorescence intensity measured by the surface detector as the inclusion depth increases. The same optical attenuation can be used to describe the decrease of CW fluorescence intensity of fluorophore with other concentrations as the inclusion depth increases.

[0049] Given the above series of experiment, the inclusion may be submerged at unknown depth and concentration in the turbid medium. As stated above, direct CW fluorescence intensity measurements alone cannot decouple the inclusion depth from its fluorophore concentration. However TD experiments can provide t_{max} which yields the inclusion depth. Once the inclusion depth is known, the calibrated optical attenuation of the turbid medium can be used to normalise the CW fluorescence intensity measurement to yield the relative concentration of the fluorophore inclusion. Further, an absolute calibration of the CW intensity measurements permits estimates of the absolute concentration.

[0050] In yet a further embodiment of the invention there is provided a method for generating a tomographic image of a fluorophore distribution. Topographic images of a region of interest can be obtained using well known method such as

CW or TD optical imaging. Such topographic images can be combined with the depth and/or concentration information obtained by the methods described above to generate a 3-dimension (tomographic) image of a volume of interest comprising one or more fluorophores.

[0051] It is also obvious to those skilled in the art that the above time domain approach can be obtained with frequency domain approach via the Fourier transform relationship.

[0052] The embodiment(s) of the invention described above is(are) intended to be exemplary only. The scope of the invention is therefore intended to be limited solely by the scope of the appended claims.

I/WE CLAIM:

1. A method for determining depth of a volume comprising a fluorophore in a turbid medium using time domain(TD) optical fluorescence, said method comprising:

   i) obtaining Temporal Point Spread Function (TPSF) data corresponding to at least one TPSF by injecting light at an injection point at an excitation wavelength of said fluorophore and detecting light at a detection point at an emission wavelength of said fluorophore and wherein said injection and detection points are in a reflection geometry and are substantially equidistant from said volume;

   ii) determining a time (t_{max}) substantially corresponding to the maximum of said TPSF;

   iii) correlating said t_{max} with said depth, wherein said depth is insensitive to fluorophore concentration.

2. The method as claimed in claim 1 wherein said step of correlating comprises:

   a) establishing a calibration curve of t_{max} vs depth for a plurality of depths;

   b) using said calibration curve and said t_{max} determined in step ii) to obtain said depth of said volume.

3. The method as claimed in claim 1 wherein said step of correlating comprises:

a) providing at least one optical property of said medium, speed of light in said medium and a lifetime of said fluorophore;

b) determining a calculated depth using said t_{max} and said at least one optical property, said speed of light and said lifetime.

4. The method as claimed in claim 3 wherein said at least one optical property is scatter coefficient.

5. The method as claimed in claim 1 further comprising a step of estimating a position of said fluorophore prior to said step of obtaining said TPSF.

6. The method as claimed in claim 5 wherein said step of estimating is performed by obtaining a topographic image of a region of interest containing said fluorophore.

7. The method according to claim 1 wherein said fluorophore is an intrinsic fluorophore.

8. The method as claimed in claim 3 wherein said at least one optical property is obtained using time domain optical measurements of said medium.

9. The method as claimed in claim 3 wherein said at least one optical property is estimated.

10. The method as claimed in claim 3 wherein said at least one optical property is obtained by matching said medium with predetermined optical properties of media in a database.

11. The method as claimed in claim 3, wherein said at least one optical property and said speed of light are substantially the same at both the excitation or emission wavelength of the fluorophore and are determined at either said emission or said excitation wavelength.

12. The method as claimed in claim 3, wherein said at least one optical property and said speed of light are determined at the fluorophore excitation and emission wavelength.

13. The method as claimed in claim 3, wherein said at least one optical property and said speed of light are determined taking into account optical properties of the turbid medium at said fluorophore location.

14. A method for estimating concentration of fluorophore in a volume in a turbid medium using optical fluorescence, said method comprising:

i) obtaining depth of said volume;

ii) providing optical properties for said medium;

iii) obtaining a CW intensity surface reflection measurement of said fluorophore; and

iv) normalizing said CW intensity measurements using said optical properties and said fluorophore depth to obtain a relative fluorophore concentration.

15. The method as claimed in claim 14 wherein said depth is obtained by the method of any one of claim 1-13.

16. The method as claimed in claim 14 wherein said optical properties are obtained using time domain optical measurements of said medium.

17. The method as claimed in claim 14 wherein said optical properties are estimated.

18. The method as claimed in claim 14 wherein said optical properties are obtained by matching said medium with predetermined optical properties of media in a database.

19. The method as claimed in claim 14, wherein said optical properties are substantially the same at both the excitation or emission wavelength of the fluorophore and are determined at either said emission or said excitation wavelength.

20. The method as claimed in claim 14, wherein said optical properties are determined at the fluorophore excitation and emission wavelength.

21. The method as claimed in claim 14, wherein said optical properties are determined taking into account optical properties of the turbid medium at said fluorophore location.

22. A method for determining concentration of fluorophore in a volume in a turbid medium using optical fluorescence, said method comprising:

i) obtaining depth of said volume;

ii) providing optical properties for said medium;

iii) obtaining a CW intensity surface reflection measurement of said fluorophore; and

iv) calibrating said CW intensity surface reflection measurement so as to obtain an absolute concentration of said fluorophore.

23. The method as claimed in claim 22 wherein said depth is obtained by the method of any one of claim 1-13.

24. The method as claimed in claim 22 wherein said optical properties are obtained using time domain optical measurements of said medium.

25. The method as claimed in claim 22 wherein said optical properties are estimated.

26. The method as claimed in claim 22 wherein said optical properties are obtained by matching said medium with predetermined optical properties of media in a database.

27. The method as claimed in claim 22, wherein said optical properties are substantially the same at both the excitation or emission wavelength of the fluorophore and are determined at either said emission or said excitation wavelength.

28. The method as claimed in claim 22, wherein said optical properties are determined at the fluorophore excitation and emission wavelength.

29. The method as claimed in claim 22, wherein said optical properties are determined taking into account

optical properties of the turbid medium at said fluorophore location.

30. A method for generating a tomographic image of a fluorophore distribution in a turbid medium said method comprising:

i) obtaining a topographic image of said fluorophore distribution;

ii) determining depth of a plurality of volumes of interest comprising said fluorophore using the method as claimed in any one of claim 1-13;

iii) combining said depth information and said topographic image to generate a tomographic image of said distribution.

31. The method as claimed in claim 30 wherein said tomographic image is further processed with the method as claimed in any one of claim 14-29 to generate a tomographic relative flurorophore concentration or absolute fluorpohore concentration image.

32. A method for determining a relative or absolute concentration of a fluorophore in a turbid medium said method comprising:

i) establishing a calibration curve relating a continuous wave intensity measurement in said turbid medium and concentration and depth of said fluorophore;

ii) determining a depth of said fluorophore using the method as claimed in any one of claim 1-13; and

iii) determining said concentration using said calibration curve.

33. A method for determining depth of a volume comprising a fluorophore in a turbid medium using time domain (TD) optical fluorescence, said method comprising:

i) providing an equation relating fluorescence and time;

ii) obtaining Temporal Point Spread Function (TPSF) data corresponding to at least one TPSF by injecting light at an injection point at an excitation wavelength of said fluorophore and detecting light at a detection point at an emission wavelength of said fluorophore and wherein said injection and detection points are in a reflection geometry and are substantially equidistant from said volume;

iii) numerically solving said equation using said obtained TPSF data to determine a predetermined time t comprised between 0 and t_{max}; and

iv) correlating said time t with said depth, wherein said depth is insensitive to fluorophore concentration.

34. The method as claimed in any one of claims 1-13, 15, 16, 23, 24 and 30-33 wherein time domain information is obtained by acquiring Frequency Domain (FD) data and applying Fourier transform to said data.

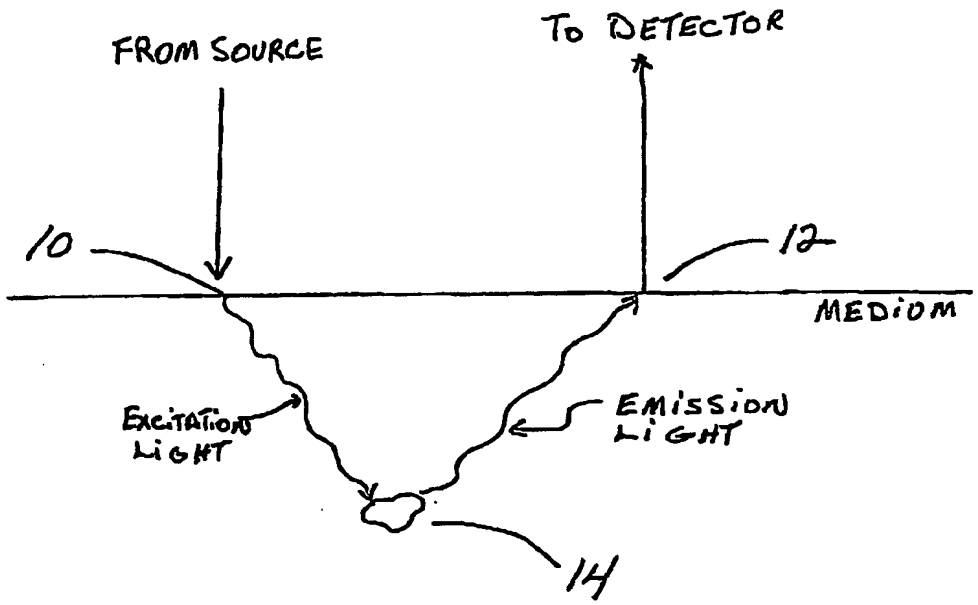

FIG. 1

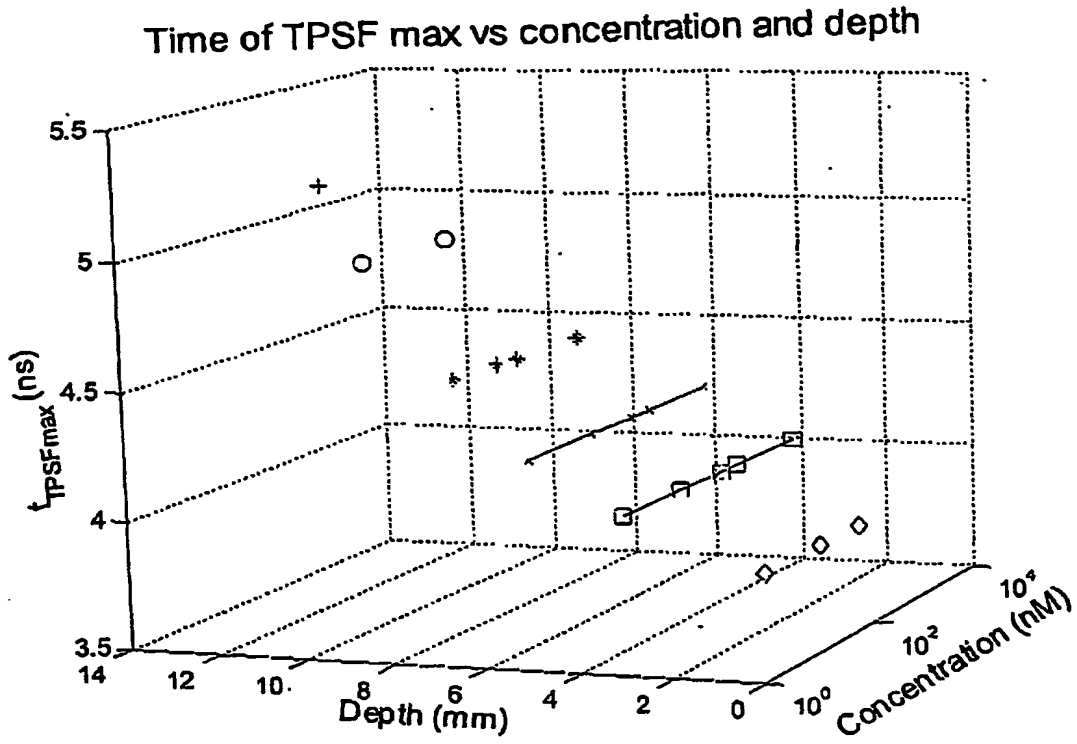
Time of TPSF max vs concentration and depth
5.5
5
4.5
4
3.5
$t_{TPSFmax}$ (ns)
14
12
10
8
6
4
2
0
Depth (mm)
10^0
10^2
10^4
Concentration (nM)

Fig. 2

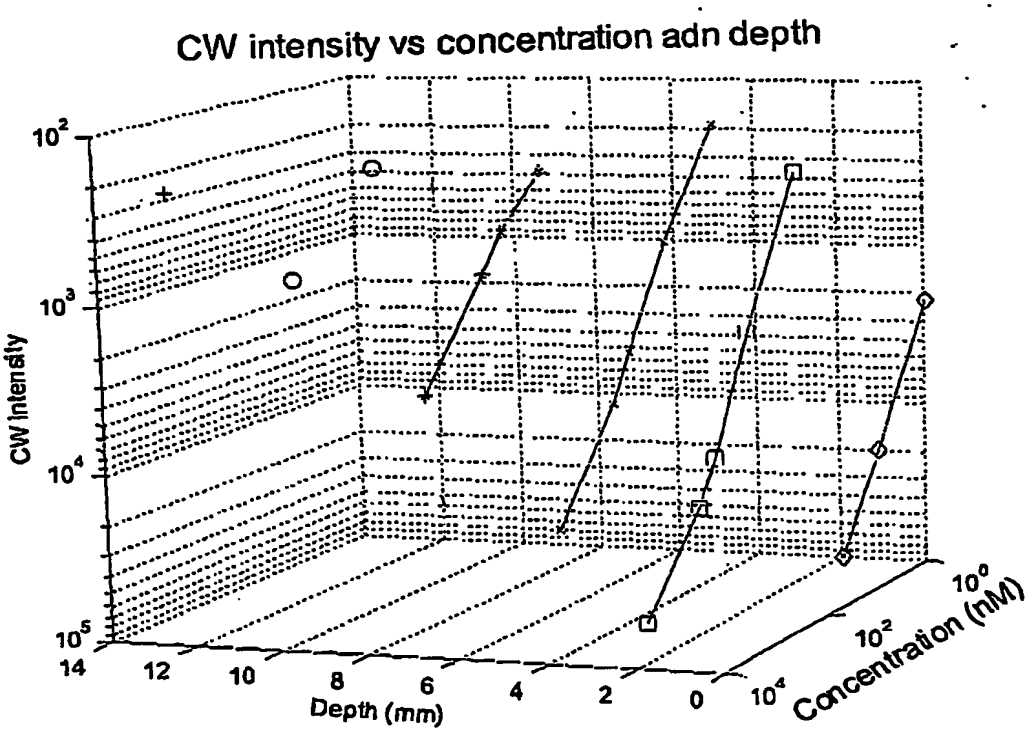
CW intensity vs concentration adn depth
CW Intensity
10^2
10^3
10^4
10^5
14
12
10
8
6
4
2
0
Depth (mm)
10^4
10^2
10^0
Concentration (nM)

Fig. 3

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/IB04/003559

International filing date: 29 October 2004 (29.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/515,697
Filing date: 31 October 2003 (31.10.2003)

Date of receipt at the International Bureau: 10 March 2005 (10.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse